Environmental Health Perspectives
Vol. 86, pp. 85–87, 1990

Metabolism and Mutagenicity of Isoprene

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Liver microsomes of various rodents (mouse, rat, rabbit, and hamster) metabolize isoprene (2-methyl-1,3-butadiene) to the corresponding monoepoxides 3,4-epoxy-3-methyl-1-butene and 3,4-epoxy-2-methyl-1-butene. 3,4-Epoxy-3-methyl-1-butene (half-life 85 min) was found to be the main metabolite, although the stable 3,4-epoxy-2-methyl-1-butene was also formed (about 14–25% with respect to the main epoxide). The kinetic constants (K_m and V_{max}) for the formation of the major epoxidic metabolite of isoprene were determined by gas-liquid chromatography. The minor epoxide was further epoxidized to the isoprene dioxide by the microsomes of all rodents studied. The K_m and V_{max} were determined and phenobarbital was found to be a good inducer for this epoxidation in all species. The mutagenic activity, using *Salmonella typhimurium*, and the chemical reactivity (alkylating power and half-life) of the epoxide metabolites of isoprene were investigated and compared to those of other structurally related epoxides. Isoprene and the monoepoxide intermediates of the isoprene biotransformation were not mutagenic in *Salmonella typhimurium*. However, the isoprene dioxide (2-methyl-1,2,3,4-diepoxybutane) was found to be mutagenic and have alkylating power towards nicotinamide, similar to the structurally corresponding 1,2,3,4-diepoxybutane. In conclusion, the metabolism of isoprene does not lead to the formation of mutagenic monoepoxide (in contrast to butadiene) but the formation of mutagenic and presumably carcinogenic isoprene diepoxide is possible, thereby a genotoxic effect of isoprene in rodents or other species cannot be ruled out.

Introduction

Isoprene (2-methyl-1,3-butadiene), an important monomer used extensively in industry and largely derived from petroleum cracking, is also the monomeric unit of naturally occurring terpenes. It is also a spontaneous product of emission from many plant species (*1*).

This paper presents our study results on the *in vitro* biotransformation of isoprene by hepatic subcellular fractions from various rodent species. Additionally, mutagenicity and chemical reactivity of epoxidic intermediates of isoprene metabolism are reported.

While the results of these biochemical and genetic observations in rodents may not be directly related to the consequences of human occupational exposure, it is hoped that they will provide some indication for future epidemiological and industrial hygiene investigations.

Metabolism

Isoprene is readily metabolized, at least in small concentrations, in rats and in mice (*1,2*) and it is partially converted into polar epoxidic metabolites. In our earlier studies, we found that the biotransformation of isoprene involves microsomal P-450-dependent monooxygenases (*3,4*).

Isoprene binds to the active site of cytochrome P-450 of microsomes from liver of rat, mouse, rabbit, and hamster resulting in Type I difference spectra showing similar K_s and ΔA_{max}. In addition, in microsomes of all rodents, different forms of P-450 appear to bind isoprene to different extents, exhibiting two K_s values and two ΔA_{max} values in the double-reciprocal plot.

Figure 1 shows the pathway of oxidative isoprene metabolism by hepatic microsomes as determined by gas-liquid chromatography (GLC) (*3*). Isoprene monoepoxides and the corresponding DIOL I and DIOL II were produced as metabolites of isoprene in the presence of O_2 and the NADPH-generating system. The classical inhibitors of P-450, metyrapone, SKF 525-A, and CO inhibit the metabolism of isoprene. In all cases

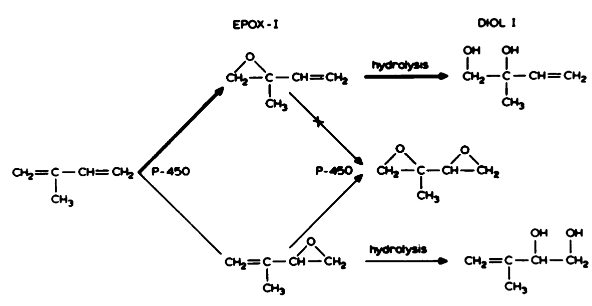

FIGURE 1. Microsomal metabolic pathways of isoprene. Major and minor metabolic pathways are indicated by thick and thin arrows, respectively.

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the oxidation resulted in EPOX-I and the corresponding DIOL I as the major metabolites. EPOX-II, which originates from the oxidation of the unsubstituted double bond of isoprene, was formed in smaller amounts from the microsomes of all the rodents examined (rabbit, 14%; hamster, 17%; mouse, 20%; rat, 25%) (*4*). In all cases the methyl-substituted double bond of isoprene is the major site for the enzymic oxidation by P-450, but not the only site. This finding is in contrast to the chemical oxidation of isoprene by peroxyacids that is selective for the methyl-substituted double bond.

Since EPOX-I was very reactive towards water (half-life 85 min), the isoprene monoepoxidase activity was determined more conveniently by following the production rate of the DIOL I after the chemical and/or enzymic hydrolysis of EPOX-I. This oxidation by liver microsomes from all rodents examined was linear over 10 min with 2 mg/mL of microsomal protein, and it followed Michaelis-Menten kinetics giving linear Lineweaver-Burk plots. The apparent K_m and V_{max} are shown in Table 1. The affinity constants did not differ significantly; however, V_{max} did vary between rats and mice by almost one order of magnitude. In mice, neither the PB nor 3-methylcholanthrene treatments were able to induce the isoprene epoxidase activity.

Liver microsomes of all animals studied were able to further oxidize EPOX-II, but not EPOX-I, to isoprene diepoxide. The hydrophilicity of EPOX-I, in addition to its high reactivity towards water and the conjugation of the double bond with the oxirane ring, probably prevents its epoxidation by P-450.

The epoxidation of EPOX-II was NADPH and 0_2 dependent, inhibited by CO, and followed Michaelis-Menten kinetics. The kinetic constants were determined by measuring the isoprene diepoxide production by GLC

Table 1. Kinetic parameters for DIOL-I formation from isoprene oxidation by various rodent microsomes.[a]

Rodent species, male	K_m, mM	V_{max}, nmole Diol-I/ mg protein × min[b]
Rat, Wistar	0.08 ± 0.05	0.24 ± 0.1
Rabbit, New Zealand	0.2 ± 0.1	0.66 ± 0.3
Hamster, Syrian golden	0.06 ± 0.04	1.20 ± 0.4[a]
Mouse, CD 1	0.09 ± 0.05	1.79 ± 0.5[a]

[a]Values are reported as an average ± SD for three or more experiments performed with different preparations of hepatic microsomes.

[b]Protein concentrations was 2 mg/mL and incubation time was 10 min.

*Significantly different from rat microsomes, $p < 0.01$ Student's *t*-test.

using microsomes from either untreated or phenobarbital (PB)-treated rodents (Table 2). Compared to controls, PB increased the V_{max} in all species examined, particularly in rats and rabbits where V_{max} was increased about 10-fold. Besides the PB induction, an important observation is that the rates of isoprene diepoxide formation were comparable to those of isoprene monoepoxidation with microsomes of untreated rodents. These findings show that the inducible formation of the reactive isoprene diepoxide could be a relevant intermediate step in mammalian isoprene metabolism.

Mutagenicity and Chemical Reactivity

Isoprene was not mutagenic in five strains of *Salmonella typhimurium*, even after metabolic activation using rat-liver microsomes (*5,6*). The mutagenic activities of isoprene epoxides and, for comparison, butadiene epoxides were tested with *Salmonella typhimurium* strains TA98 and TA100 (*7*). All epoxides were assayed with the standard-plate incorporation test, without metabolic activation since they are direct-alkylating compounds. Among the epoxide metabolites of isoprene only the diepoxide proved to be mutagenic in TA100 strain with a linear dose-effect relationship, while the structurally related butadiene epoxides all showed mutagenic activities in TA100, in agreement with published data (*8*).

To study the correlation between mutagenic activities of oxiranes and their chemical properties, Table 3 shows: the mutagenic activity of isoprene and butadiene epoxides at 0.15 mM concentrations, measured from the linear part of the dose-effect relationship; the half-lives of epoxides in Tris-buffer, pH 7.4, determined by a GLC; and the alkylation rates of epoxides towards nicotinamide, a nucleophilic target (*9*). EPOX-I the main metabolite of isoprene, was neither mutagenic nor alkylating although its reactivity towards water was high.

By contrast, the corresponding epoxide without the methyl group in the oxirane ring, 1,2-epoxy-3-butene, although less reactive towards water than EPOX-I, was an active alkylating agent and mutagenic to Salmonella. A similar effect of methyl as a substituent in the oxirane ring has been found for other compounds. α-Methyl styrene oxide, 2-methyl propylene oxide, and 1,2-epoxy-2-methylbutane did not show mutagenic activity (*10*) while the corresponding styrene oxide, propylene oxide and 1,2-epoxybutane were mutagenic and had alkylating activity (*7*). The methyl substitution in the oxirane ring causes a steric hindrance. Because of its

Table 2. Effect of PB pretreatment of rodents on the microsomal oxidation of EPOX-II.[a]

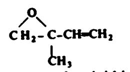
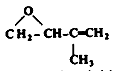
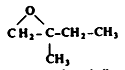
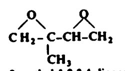
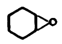
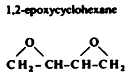
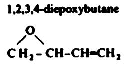
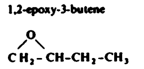
	Hamster		Rat		Rabbit		Mouse	
Kinetic parameters	Control	PB	Control	PB	Control	PB	Control	PB
K_m, mM	0.9 ± 0.2	1.9 ± 0.7	ND	0.6 ± 0.2	ND	5.9 ± 2.1	0.24 ± 0.03	0.29 ± 0.05
V_{max}, nmole diepoxide/mg protein × min[b]	1.5 ± 2.3	5.6 ± 1.2	0.3 ± 0.1	3.8 ± 1.3*	0.2 ± 0.1	4.21 ± 1.5	1.7 ± 0.4	5.1 ± 1.2*

[a]Values are means ± SD of three experiments performed with different preparations of hepatic microsomes.

[b]Protein concentration was 2 mg/mL and incubation time was 15 min.

*Significantly different from control microsomes of the corresponding animal species ($p < 0.01$, Student's *t*-test).

Table 3. Mutagenicity (Ames test S. *typhimurium* TA 100) alkylation rates (nicotinamide-test) and half-life of epoxides.

Compound	Alkylation rate, fluorescence/hr	Mutagenicity, revertants/plate*	Half-life, hr
 a. 3,4-epoxy-3-methyl-1-butene	15	0	1.25
 b. 3,4-epoxy-2-methyl-1-butene	35	0	73
 c. 1,2-epoxy-2-methylbutane	10	0	112
 d. 2-methyl-1,2,3,4-diepoxybutane	360	1700	46
 e. 1,2-epoxycyclohexane	30	153	128
 f. 1,2,3,4-diepoxybutane	400	1366	100
 g. 1,2-epoxy-3-butene	180	346	13.7
 h. 1,2-epoxybutane	150	257	156

* Histidine^{+} revertants induced by 15 mM epoxide

electron-donating action, the methyl group could effect the mechanism of nucleophilic substitution, shifting the substitution mechanism towards an SN_1 type. In such a condition, the highly polarized carbon atom, partly hampered by the methyl group, will react mainly with small polar molecules such as water. The correlation between mutagenicity and alkylating power for epoxides holds when the mechanism of alkylation is an SN_2 type, but not when it becomes mainly SN_1. This correlation was shown for the carbocationic intermediates of ethylating or methylating mutagenic agents (*11*).

The lack of mutagenicity and alkylating abilities of EPOX II could be explained by the fact that the methyl substitution on the double bond causes a steric effect and, moreover, can counteract the oxirane destabilization because of the resonance between double bond and oxirane ring.

Isoprene diepoxide was found to have a half-life and mutagenic and alkylating activities similar to those of the structurally related butadiene diepoxide, a mutagenic, clastogenic, and carcinogenic compound.

Conclusions

Isoprene metabolism showed the same pattern in all rodent species studied; in contrast to the metabolism of the analogs butadiene (*12*) or other conjugated olefins (*13*), it did not result in mutagenic monoepoxide intermediates.

The ratios between the isoprene monoepoxides formed were similar in all rodents studied. Although only 3,4-epoxy-2-methyl-1-butene, the monoepoxide produced in small amount, was able to be further oxidized by P-450-dependent monooxygenases to the mutagenic isoprene diepoxide, a possible mutagenic and/or carcinogenic potential of isoprene still remains. However, investigators need to clarify whether a similar metabolic pattern occurs in extrahepatic rodent tissues and, more important, in human tissues and in other living species.

This work was supported in part by Progetto Finalizzato "Oncologia" of C.N.R.

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